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Regeneration in Sphaerocarpos Donnellii

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(WITH TWENTY-FIVE TEXT FIGURES)

Regeneration, or the production of adventitious shoots from the vegetative tissue, has been described in *Sphaerocarpos* by Leitgeb (4), C. and R. Douin (2), and Goebel (3). Leitgeb describes regeneration in *S. Michelii* Bell. (*S. terrestris* Sm.) as occurring from both the lobes and the midrib of the thallus. It results from the growth of a single cell into a cylindrical body, whose tip gradually broadens out and becomes flat, forming the normal plant body. As in the case of sporelings, sex organs are formed very early—in some cases before the young shoot has assumed the flat shape.

The Douins (2), studying *S. Michelii* and *S. texanus* Aust. (*S. californicus* Aust.), observed regeneration taking place from the midrib and from the involucres, and state that it probably occurs also from the lobes, though this was not seen by them, Regeneration is most marked in connection with the female thalli. When these are destroyed by drought, the midrib remains alive for some time, and if a period of humidity follows, each fork of the midrib elongates, forming more or less numerous lobes and involucres. Regeneration also occurs from the involucres, when these are accidentally bent over and brought into contact with the soil. Certain cells of the involucre in these cases multiply and give rise to new thalli. The Douins' figures show such plants arising from around the opening of the involucre, in exactly the same way as is illustrated in my FIG. 18. The regenerated thalli are somewhat different from the old ones, especially in the case of the male, showing dorsal lobes, whose form leads the authors to conclude that they are modified involucres.

Goebel (3), working with *S. Michelii* (possibly mixed with *S. texanus*, if the latter species is found throughout Europe as it is in France), found that regeneration occurs when portions of the thallus, especially the involucres, are broken off or partly broken off, and are in contact with the soil. Regeneration is most easily observed if separated portions of the involucres are cultivated in water. Shoots may be produced either on the inside or on the outside of the involucre, and rhizoids are produced at the same time, especially in the neighborhood of the shoots. When shoots develop on the inside of the involucre, it is necessary for them to curve about so as to emerge; if formed on the outside of the involucre, they grow straight outward. A shoot is formed as a cell mass arising from a single cell. At the tip of this body is formed the growing point. Right and left from this, flat wings develop. Sometimes a long germ-tube is first formed, consisting of many cells. In such a case, the whole structure resembles a sporeling, except that the formation of the flat plate at the end of the germ-tube is not at right angles to the germ-tube, but simply a spreading out and flattening of the latter. The history, except in a few cases, is not so simple as that described by Leitgeb, the wings not necessarily lying in one plane, and the formation of the flat plate resulting from the spreading out of the first cylindrical body. Sometimes one of the wings goes on to develop into a thallus, apparently because the development of the growing region between the two wings was arrested. Goebel compares the formation of these adventitious shoots with spore germination, and emphasizes the fact that they may develop either almost exactly as does a sporeling, namely by the formation of a flat plate of cells from the terminal group of cells of the germ-tube, or by a direct and simple flattening out of the primary cell mass, which fact shows of how little value is any sharp distinction between these two methods in a discussion of spore germination.

I first noticed the occurrence of regeneration in searching for germinating spores in soil cultures of *S. Donnellii* Aust. Many small plants which I picked up proved to have no spore wall attached at the basal end, and in some cases were found instead to be attached to old dead portions of tissue. In these cases the plants were of a ribbon-like form, broadening out a little at the anterior end. In cultures made later for the purpose of obtaining regeneration, the young plants were broader and less ribbon-shaped. The light conditions under which these latter plants had developed were more favorable. In the cases first noted the young plants were growing among older plants, which overshadowed them to a large extent, and the elongated ribbon form is probably a resultant of these conditions.

Regeneration also occurred from the involucres of several female plants which were being cultivated singly on soil. Conditions here were such as to produce the extreme leafy form of the thallus described in a previous paper (5); as the posterior part of the thallus died, small masses of cells protruded from the involucres in this region and later grew out into young thalli.

Attempts were made to induce regeneration by artificial means, such as the wounding of the female thalli. In cases in which involucres or lobes were almost or completely separated from the plant body, regeneration occurred abundantly, though not usually at points showing any definite relation to the cut surfaces. The rest of the plant continued to grow in a normal manner, although occasionally adventitious shoots were formed on the midrib, often near places where portions had been cut off. I have never observed regenerated shoots to arise directly from one of the wounded surfaces. Very frequently rhizoids developed from the cut end of the separated portion of the thallus; these appeared without any spatial relation to the adventitious shoots and sometimes before the latter became evident.

Attempts were also made, in every case unsuccessfully, to induce regeneration, using the same or similar methods, from the sex organs, and also from young sporophytes.

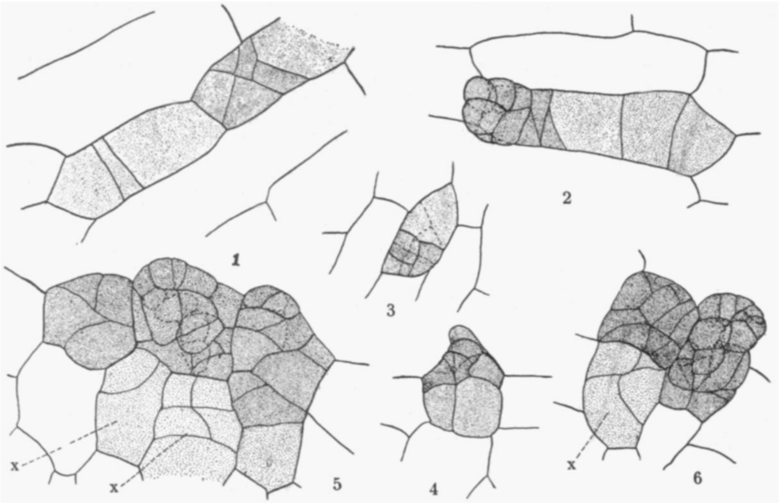
The occurrence of regeneration is frequent in cultures, especially from the female thalli, and seems to be a regular method of vegetative multiplication, rather than merely a response to abnormal conditions. After the development of sporophytes, the mother plants often die in large part, and growth continues after a time by means of regeneration from their surviving portions. C. Douin (1), describing the life history of *S. Michelii* under natural conditions in Europe, states that the old thalli are usually buried by the tilling, and that the spores produced on these plants germinate with the spring rains. If regeneration occurs as abundantly under natural conditions as it does in cultures, it seems not impossible that the old thalli thus buried in the spring may continue their growth by the production of adventitious shoots.

My observations seem to warrant the conclusion that regeneration is a rather common phenomenon in *Sphaerocarpos*, resulting from particular nutritive conditions in the thalli or in parts of the thalli, rather than merely from a direct response to occasional accidental stimuli.

The descriptions that follow are based on data obtained from female plants, these being more abundant and more convenient for study than the male. In a few male plants that were studied, the phenomena observed were similar to those here described. The plants produced by regeneration were in all cases of the same sex as the parent plant. Several cultures of female plants, which were started by sowing involucres separated from the thalli, on soil, are still growing vigorously at the present time, nearly twelve months afterwards, and are still purely female.

In order to study in detail the method of formation of the adventitious shoots, involucres, lobes, and small portions of the midrib were cut off and placed on moist filter paper in petri dishes. At the end of two weeks or thereabouts (in one case after six days), these small pieces of tissue were covered with outgrowths in various stages of development. They were then removed, killed by placing in a dilute solution of chrom-acetic acid (chromic acid, 0.3 g.; glacial acetic acid, 0.7 cc.; distilled water, 99 cc.), stained in eosin, and mounted in glycerin.

When regeneration is about to occur, the contents of a single cell become more dense and richer in chlorophyll content. Such a cell may be isolated, or there may be a group of similar cells adjoining or in close proximity to one another. Cell division then occurs, without an accompanying growth of the mother cell. The relative position of the walls formed in these divisions is quite variable. FIGS. 1, 2, 3, 4, 13, and 14 represent groups of cells formed in this way. It makes no particular difference whether the original cell was on the edge or in the middle of the tissue of the parent plant, nor does its size or shape have any influence on the course of development. Very commonly, indeed in the majority of cases, the walls are set in at acute angles to each other, and in the case of the involucral cells these divisions occur mostly at one end of the mother cell, the other end being divided into only two or three comparatively large and clear cells (FIGS. 1, 2). The end cell of a row of cells formed in this way suggests a wedge-shaped apical cell, cutting off segments alternately to right and to left. The process is, however, too variable to justify the conclusion that the growth of adventitious shoots is regularly due at first to the activity of such an apical cell.

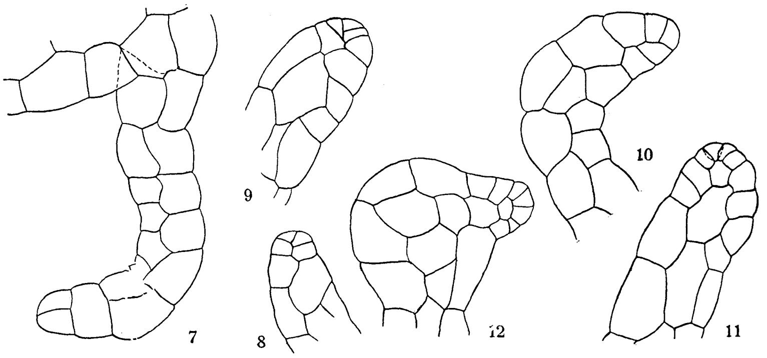


FIGS. 1–6. Single involucral cells dividing to form adventitious shoots. The shading indicates the extent of the original mother cells, the darker portions representing those of the densest contents. FIG. 1. Two adjacent mother cells at an early stage of regeneration. FIGS. 2, 3. The young shoots beginning to project above the surface of the parent tissue. FIG. 4. Shoot arising from a marginal mother cell. FIGS. 5, 6. Shoots arising from adjoining marginal mother cells: *x*, *x*, adjacent involucral cells also dividing. FIGS. 1–4, 6, × 150; FIG. 5, × 200.

As cell division continues, the cell mass so formed begins to protrude above the surface of the parent tissue at one end of the mother cell (FIG. 2). This seems to be due simply to a bulging up of the mass of cells, followed by cell divisions in planes more or less nearly parallel to the surface of the tissue from which the shoot is growing. If regeneration occurs from the margin of the parent tissue instead of in the middle, this bulging out commonly does not occur; the young plant grows straight out from the margin, and gradually curves so as to bring its distal portion into a more erect position.

After the young plant has thus become a distinct outgrowth from the parent tissue, cell divisions occur in many planes, so that there is formed a compact mass of cells several cells thick in each dimension. The form of this mass is globular or cylindrical, and there is no longer any evidence of the existence of an apical cell (FIGS. 5, 6).

The only exceptions found to this history were in the first cases of regeneration noted, mentioned above, in which each young plant grew out as a long ribbon. Presumably this took place by the continuation, after the cell mass had once bulged out from the original containing wall, of divisions similar to those which had cut up the original mother cell. The cell at the tip of such a ribbon-shaped growth had in most cases exactly the appearance of a two-sided apical cell, cutting off segments alternately to right and to left. The segments themselves redivided only occasionally, so that a narrow shoot, two cells wide and one cell thick, resulted. The tips of such shoots are illustrated in FIGS. 7, 8, 9, 10. These young plants finally broadened out at the tip (possibly in response to more favorable light conditions), and the ordinary flat plate-shaped thallus resulted. As soon as the broadening had occurred, an apical cell could no longer be distinguished, further growth being due to a group of small meristematic cells on the margin of the plate, either in an apical or in a lateral position (FIGS. 11, 12, 19).

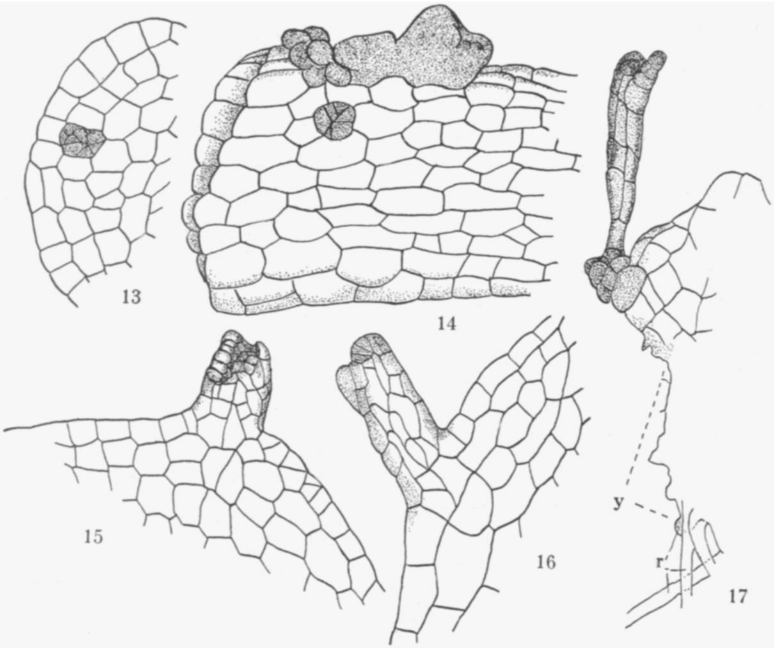


FIGS. 7–12. The tips of filamentous adventitious shoots. FIGS. 8–10. Development by means of a two-sided apical cell. FIGS. 11, 12. Broadening of the apical region into the thallus-plate. All, × 150.

This history bears a striking resemblance to that of the sporelings described in a previous paper (5), which developed from spores sown in a nutrient solution. These sporelings were marked by the production of an excessively long germ-tube, which, when conditions were favorable, gradually broadened out into the thallus-plate. In the cases of some sporelings (5, *f. 35*), ribbons were formed which were almost identical in appearance with those just described as arising by regeneration. Obviously the unusual form in each case bears much the same relation to the normal or common method of development. All gradations are found between the two methods among both sporelings and adventitious shoots, the latter, however, being, so far as my observations have shown, by far the more variable.

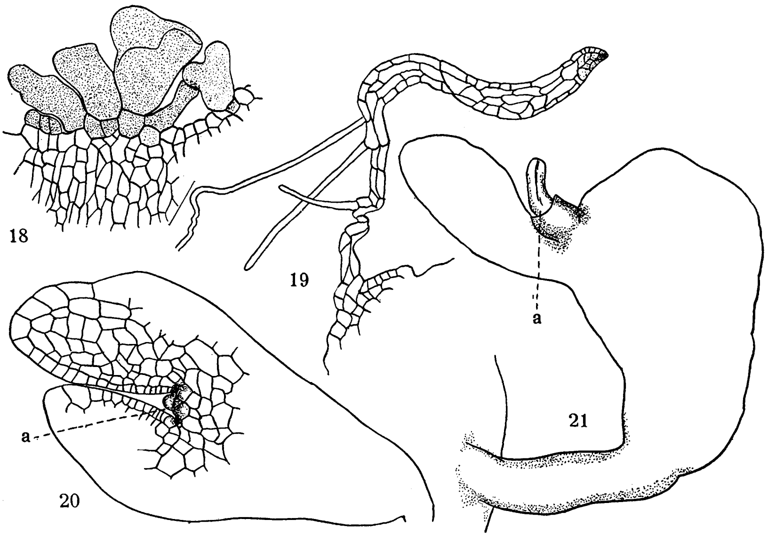
In cases in which several adjoining cells start regeneration, it seems that the growths produced by each may become fused together into one new plant. This is rather difficult to determine, but it is certain that one often sees many contiguous cells starting to divide, while there are rarely as many plants formed on a single portion of tissue as would represent the development of all these cells into separate plants. The possibilities suggested are that fusion may occur, two or more cell masses growing together into one structure, or that the development of some of these cell masses may be checked. FIG. 14 shows adventitious growths resulting from the division of groups of adjacent cells. In FIGS. 15, 16, and 19, the young plants seem to have developed each from a single marginal cell of a lobe, the base of the shoot not being much wider than a single marginal cell. FIG. 18 represents several plants arising by regeneration from the margin of the opening of an involucre. Here also each plant seems to have developed from a single cell. FIGS. 5 and 6 show similar groups of marginal cells at an earlier stage in regeneration. The cell masses resulting from the division of individual marginal cells can be fairly readily distinguished, and seem to be each in process of forming independent shoots; but there are other cells (x) adjoining these which have also divided, and it is impossible to say whether the cell groups thus formed will also give rise each to a separate plant or whether they will merely contribute to the cell masses formed from the marginal cells.

Very often, the surrounding cells being largely dead or dying, the cells which form the new plants assume a globular form and stand out from the surrounding tissue. This is especially true when regeneration occurs from the midrib. Such a case is shown in FIG. 17. Here also there is a strong suggestion that a group of cells is concerned in the production of a single new plant.



FIGS. 13–17. The further development of shoots produced by regeneration from involucres and lobes. The shading indicates the density of the cell contents in the new plants. FIG. 13. Regeneration from a single cell of a lobe. FIG. 14. Cell masses produced by the division of a number of adjoining mother cells of an involucre. The cell contents were so dense that the cell walls could not be distinguished. FIGS. 15, 16. Regeneration from the margins of lobes. FIG. 17. Regeneration from the midrib: *y*, cut surface; *r*, rhizoids. All drawn from living material, × 75.

In the course of the usual history, after the young plant has developed into a small globular or cylindrical mass, a flat plate is formed as the result of the continued division of a group of cells at the tip of the structure. FIGS. 15 and 16 represent cases in which this process is taking place at an early stage; in FIGS. 17, 21, 22, and 23 the original cell mass has developed into a long and comparatively slender body, in one case (Fig. 17) strongly suggestive of the germ-tube formed as a result of spore germination. As in the latter process, the thallus-plate is at first usually cup-shaped or trough-shaped (Fig. 15), and the growing point may lie in a median or in a lateral position. The tissues formed to right and left of the apical point grow rapidly, so that the latter lies at the base of a deep apical notch (Figs. 20, 21, 22). As in the case of the sporeling, the first sex organ appears very early (Fig. 21), being formed immediately behind the growing point. Presumably an apical cell, or group of initials, is developed in the growing region just previous to the formation of the first sex organ, since from this point the history of the adventitious shoot is identical with that of a typical thallus (5).



Figs. 18–21. Young plants produced by regeneration. Fig. 18. Shoots arising from a number of adjoining marginal cells of the involucre. The cellular structure of the shoots is not shown. Fig. 19. Filamentous shoot produced from the margin of a lobe. Fig. 20. The terminal plate of a young plant, showing the apical notch: *a*, young archegonium. Fig. 21. Young shoot, consisting of a cylindrical basal portion and a terminal flat plate: *a*, archegonium enclosed in young involucre. Figs. 19–21 drawn from living material. Figs. 18, 19, × 35; Figs. 20, 21, × 75.

In the case of some of the male plants studied, the young plant arising by regeneration produced only a few antheridia, and assumed a very elongated form, with few lobes. Such a plant is shown in FIG. 23. Similar results were obtained from female plants by allowing regeneration to occur under water (FIGS. 24, 25). In one case (FIG. 24), no sex organs were produced, and scarcely any lobes. In another case (FIG. 25), the tip of the plant reached the surface of the water and there began to take on more nearly the ordinary appearance. Production of rhizoids was especially abundant in this latter case.

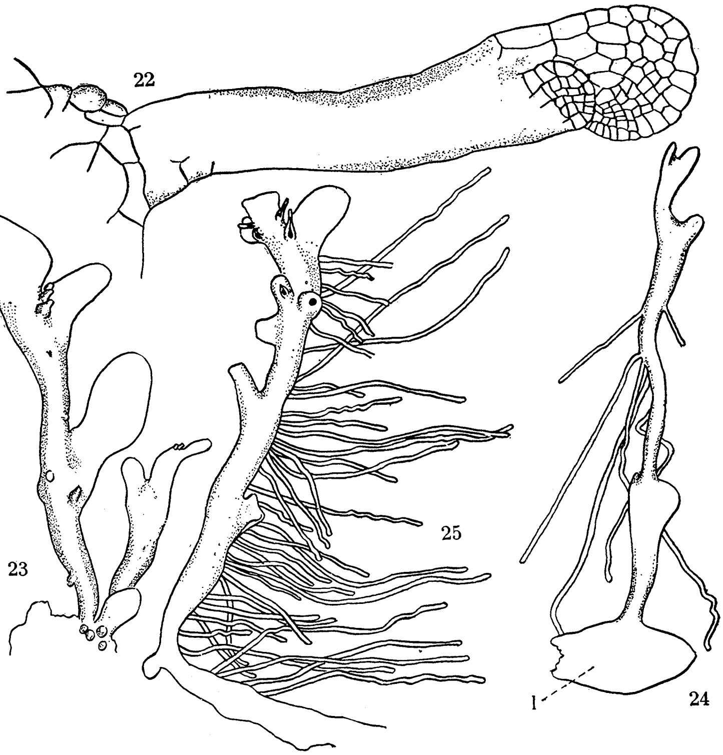


FIG. 22. The apical notch formed laterally on a long cylindrical cell mass. FIG. 23. Young plant, with antheridia, produced by regeneration from the lobe of a male plant. FIGS. 24, 25. Shoots developed from portions of female plants cultivated under water: *l*, parent lobe. All drawn from living material. FIG. 22, × 100; FIGS. 23–25, × 20.

SUMMARY

1. Regeneration in *Sphaerocarpos Donnellii* may occur from any vegetative tissue of the thallus, either when the thallus as a

whole is partly dead, or when portions of it are separated or partly separated from the rest under conditions favorable for growth.

2. Plants produced by regeneration originate for the most part in a single cell, which divides irregularly to form a cell mass whose shape is dependent on environmental conditions. It is possible that in some cases this cell mass may originate from several adjoining cells of the parent plant instead of from one cell only.

3. There is a tendency towards the formation of a two-sided apical cell in the early stages, this being most marked when conditions are comparatively unfavorable. This tendency may lead to the formation under unfavorable conditions of a long, narrow, ribbon-shaped plant.

4. The development of the first cell mass, globular, cylindrical, or ribbon-like, into a typical thallus takes place in a manner entirely analogous to the development of a mature thallus from the germ-tube produced by a spore on germination.

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